1. Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common malignant tumors in the head and neck, which mainly affects middle-aged and elderly people aged 40 to 60 years. The incidence of OSCC is higher in men than in women. This cancer can occur in any part of the mouth, but one of the most common is tongue cancer, which usually manifests on both sides of the tongue. [1] In addition, gingival cancer, buccal mucosa cancer, palate cancer and maxillary sinus cancer are also common types. Early symptoms may include abnormal spots or ulcers on the tongue or other affected areas, which may be difficult to heal. [2] The five-year survival rate of oral squamous cell carcinoma (OSCC) is less than 60%, [3] reflecting its severity and treatment challenges. The survival rate of this cancer is influenced by several factors, the most important of which is the stage of cancer discovery. Early detected cases usually have higher cure and survival rates, but many cases have progressed to advanced stages by the time they are diagnosed because early symptoms may not be obvious or easily overlooked. In addition, the patient's age, overall health status, and the specific location of the cancer are also important factors affecting survival. For example, cancers in areas of the mouth that are less visible, such as deep in the throat, may be more difficult to detect early. [4] Treatment methods include surgical resection, radiotherapy and chemotherapy, but the success of treatment and the prognosis of patients largely depend on the type, location and stage of cancer development. Due to the complexity of this cancer, comprehensive treatment planning and early diagnosis tailored to each patient are key to improving survival. Leukocyte differentiation antigen 109 (CD109) is a cell surface glycoprotein that is attached to the cell membrane by a glycosyl phosphatidylinositol (GPI) -anchoring mechanism. [5] The structure and function of CD109 enable it to play an important role in a variety of biological processes, especially in the regulation of the immune system and the development of certain diseases. In the field of oncology, the expression level and pattern of CD109 may be related to tumor development, invasiveness, and patient prognosis. [6] Therefore, it is being investigated as a potential biomarker to aid in diagnosis, monitoring of treatment effects, and predicting clinical outcomes in certain cancers. [7] As a coreceptor of transforming growth factor-β (TGF-β), CD109 inhibits Tgf-β signaling pathway by regulating the endocytosis and degradation of Tgf-β receptor [8], thus affecting the occurrence and development of tumors. In this study,
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2. Materials and Methods

2.1. Access to bioinformatic information

The online TIMER2.0 database (http://timer.compbio.i犁.org/) was used to detect the expression level of CD109 mRNA in various cancer tissues. CD109 mRNA expression in tumor and normal tissue data from UALCAN head and neck squamous cell carcinoma of the database (http://ualcan.path.uab.edu/analysis.html/), the database is based on the TCGA sample. A total of 520 primary head and neck squamous cell carcinoma tissues and 44 normal tissues were included.

2.2. Study design

Wax blocks were selected from the pathology center of our unit, and the time span was from January 2015 to January 2023. Of these, 20 were normal oral mucosal epithelium and 75 were OSCC (including 24 tongue cancer, 23 buccal cancer, and 28 gingival cancer), and adult patients aged between 26 and 80 years were selected. There were 40 males and 35 females, 58 cases without lymph node metastasis and 17 cases with lymph node metastasis. Twenty-three cases were poorly differentiated and 52 cases were moderately or well differentiated. Inclusion criteria: 1. Primary tumor located in the oral cavity; 2. Preoperative diagnosis by two experienced pathologists independently; 3. No adjuvant therapy of radiotherapy or chemotherapy was performed before surgery.

The specimens were fixed in 10% neutral formalin, routinely dehydrated and embedded in paraffin, and 4μm thick sections were prepared, stained with HE, and observed by light microscopy. The antibody kit was purchased from Fuzhou Maixin Biotechnology Development Co., LTD. (Fuzhou, China). Rabbit anti-human CD109 polyclonal antibody was used. Immunohistochemical staining was performed using Roche's EnVision two-step method. Detection was performed using the CD109 antibody and was performed according to the experimental procedure, with known positive tissues selected as positive controls and PBS used instead of the main antibody as negative controls. This study was approved by the ethics committee. Signed written informed consent were obtained from the patients and/or guardians.

2.3. Evaluation of staining results

Scoring was performed independently by two experienced pathologists blinded to clinicopathological information. Ten fields were randomly selected for each section, and 100 cells were counted in each field. The final IHC staining score was calculated by multiplying the percentage score and the intensity score. The score was 0 for unstained, 1 for light yellow staining, 2 for brown-yellow staining, and 3 for dark yellow staining. A score of 0 was given if less than 5% of the cells were positive. The positive cells between 5% and 25% were scored as 1 point. The positive cells between 25% and 75% scored 2 points. The positive cells were 75% or more, and the score was 3. A score of 0-2 indicates negative expression and a score of 3 or greater indicates positive expression.

2.4. Statistical analysis

Statistic Package for Social Science (SPSS) version 22.0 statistical software (IBM, Armonk, NY, USA) was used for statistical processing, contingency tables were used to compare the count data between groups, and Chi-square test or Fisher's exact probability method was used. P<0.05 was considered statistically significant.

3. Results

3.1. The analysis of TIMER2.0 database

Through the TIMER2.0 database, pan-cancer analysis found that the expression of CD109 mRNA was up-regulated in 14 cancer tissues compared with normal tissues (including head and neck squamous cell carcinoma) (the more * symbols, the greater the difference) (Figure 1). A total of 520 primary head and neck squamous cell carcinoma tissues and 44 normal tissues were studied using the UALCAN database, and CD109 mRNA expression was significantly higher in head and neck squamous cell carcinoma tissues than in normal tissues (p<0.01, Figure 2).

3.2. The expression of CD109 in normal oral mucosa and oral squamous cell carcinoma

The expression of CD109 was significantly higher in both benign and malignant differentiated oral squamous cell carcinoma tissues than in normal oral mucosa tissues (Figure 3A-C). There was a significant difference in the positive expression rate of CD109 between normal oral mucosa group and oral squamous cell carcinoma group (P<0.05, Table 1).

3.3. Clinical and pathological features

The expression rate of CD109 in lymph node metastasis group was 94%, and that in non-lymph node metastasis
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for about 3.3% of the genome sequence, a relatively large proportion indicating a complex gene structure [10]. The GPI-anchoring mechanism is a general way of anchoring proteins to the cell membrane. In the case of CD109, the GPI anchor allows this glycoprotein to be firmly localized on the cell surface and thus efficiently participate in cell-to-cell communication and signaling. This localization is essential for maintaining normal cellular function and regulating immune responses [11]. From a functional point of view, CD109 may play different roles in different cell types and biological processes. In the immune system, CD109 may be involved in regulating the activity of T cells and other immune cells. Furthermore, in terms of tumor biology, CD109 expression and function have been found to be of potential importance in the development of certain cancers. It may be associated with the proliferation, migration and invasion of tumor cells [12]. Overall, the expression pattern, function, and association with disease of CD109 make it an important target for biomedical research, especially in the fields of oncology and immunology. However, further studies are needed to fully understand its role in complex diseases [13].

Studies have shown that CD109 is highly expressed in squamous cell carcinoma of multiple sites, such as lung, esophagus, cervix, skin, etc. [14-16], and CD109 is low or not expressed in most normal human tissues [17]. This study also confirmed that CD109 is highly expressed in oral squamous cell carcinoma, and its expression is very low in normal oral tissues. Previous studies have found [18] that CD109 plays a role in the transformation of precancerous lesions of oral mucosa into oral cancer. The results showed that CD109 was highly expressed in oral squa-

4. Discussion

CD109 is a glycoprotein anchored to the cell surface by glycosyl phosphatidylinositol (GPI). This protein plays a key role in a variety of biological processes in humans, including cell signaling, immune regulation and, in some cases, association with disease development [9]. At the molecular level, the structural features of CD109 are remarkable. Its gene is located on human chromosome 6 (specifically the 6q region) and consists of 33 exons. Together, these exons encode the amino acid sequence that forms the CD109 protein. The CD109 gene accounts

Table 1. Expression of CD109 in normal oral mucosa and oral squamous cell carcinoma.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>The positive expression of CD109 (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal oral mucosa</td>
<td>20</td>
<td>3 (15)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Oral squamous cell carcinoma</td>
<td>75</td>
<td>48 (64)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Relationship between CD109 expression and clinicopathological features of patients with oral squamous cell carcinoma.

<table>
<thead>
<tr>
<th>Clinicopathologic feature</th>
<th>n</th>
<th>The positive expression of CD109 (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Male</td>
<td>40</td>
<td>27 (68)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>35</td>
<td>21 (60)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>&lt;60y</td>
<td>39</td>
<td>28 (72)</td>
<td></td>
</tr>
<tr>
<td>≥60y</td>
<td>36</td>
<td>20 (56)</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>&lt;2 cm</td>
<td>30</td>
<td>21 (70)</td>
<td></td>
</tr>
<tr>
<td>≥2 cm</td>
<td>45</td>
<td>27 (60)</td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gingiva</td>
<td>28</td>
<td>18 (64)</td>
<td></td>
</tr>
<tr>
<td>Tongue</td>
<td>24</td>
<td>17 (71)</td>
<td></td>
</tr>
<tr>
<td>Cheek</td>
<td>23</td>
<td>13 (57)</td>
<td></td>
</tr>
<tr>
<td>Lymphatic metastasis</td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>16 (94)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>58</td>
<td>32 (55)</td>
<td></td>
</tr>
<tr>
<td>Differentiated degree</td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>High school differentiation</td>
<td>52</td>
<td>26 (50)</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>23</td>
<td>22 (91)</td>
<td></td>
</tr>
</tbody>
</table>
mous cell carcinoma, which was significantly different from that in normal oral mucosa (P<0.05). The expression rate of CD109 in poorly differentiated OSCC was 91%, which was higher than that in well-differentiated OSCC (22%). There was a significant difference between the two groups (P<0.05), indicating that the expression of CD109 increased with the malignant degree of oral squamous cell carcinoma. It can be speculated that CD109 plays an important role in the occurrence and development of oral squamous cell carcinoma. This study found that the expression of CD109 was not correlated with the patient’s age, gender, tumor size, and oral tumor location, but the high expression of CD109 was more common in patients with lymph node metastasis. Moreover, it has been shown that high expression of CD109 inhibitors can impair the migration and invasion ability of OSCC cells. Thus, the risk of OSCC metastasis can be reduced [19,20]. Therefore, it can be boldly speculated that CD109 may be involved in the metastasis process of OSCC.

In conclusion, the expression of CD109 in OSCC is significantly increased compared with normal oral mucosa, and it plays an important role in the occurrence, development and metastasis of OSCC. The higher the positive expression of CD109, the higher the possibility of lymph node metastasis. Therefore, the high expression of CD109 can be used as a biological marker for predicting the occurrence and lymph node metastasis of OSCC.

Conflict of Interests
The author has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
This study was approved by the ethics committee of Ningbo Women and Children’s Hospital.

Informed Consent
Signed written informed consents were obtained from the patients and/or guardians.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions
HC and JX designed the study and performed the experiments, RG collected the data, CL analyzed the data, HC prepared the manuscript. All authors read and approved the final manuscript.

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